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Development of a biological phosphate fertilizer to improve wheat (Triticum aestivum L.) production in Mali.

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Abstract

Tilemsi Rock phosphate Tilemsi (TRP), is a good and inexpensive alternative to imported phosphate fertilizers. Thiobacillus thioparus, Thiobacillus thiooxidans and Thiobacillus ferooxidans, are known for their ability to oxidize soil sulfur and sulfides and influence the solubilization of inorganic phosphates. These bacteria have also a good potential to improve plant growth. In order to improve wheat response to fertilization with TRP, we describe in this work, the isolation from agricultural soils and the selection of different Thiobacillus strains with high TRP-solubilizing activities. Initially, 91 acidifying bacteria were selected, but after 10 subcultures on agar and in liquid media, only four bacteria were chosen for their strong P-solubilizing activities. No Thiobacillus ferrooxidans, was isolated from tested agricultural soils. A significant correlation was observed between acid production by isolated strains and phosphate solubilization. In a field trial in Koygour (Dire), wheat (Triticum aestivum cv. Tetra) was inoculated with selected strains of Thiobacillus and fertilized with the TRP or diammonium phosphate (DAP). The growth and yield parameters measured were the plant the tillers per plant, panicles per plant, percentage of fertile tillers (number of tillers with panicles/total number of tillers), panicle length, grain per panicle, grain yield calculated at 13% moisture, and 1000 grain weight. Yield increase relative to control and yield relative efficiency were also calculated. BioTRP1, with yield of 2840 kg/ha grain, increased grains and straw yield at 45.64 and 73.81%, respectively with the highest relative yield efficiency. BioTRP1 is the only treatment not significantly different from the Di-ammonium phosphate. At harvest, average number of tillers was 18 for treatment 5 and a bit lower for di-ammonium phosphate with 17.8. Percentage of fertile tillers was 92% and 90% for the di-ammonium phosphate and the BioTRP1 treatments respectively. Panicle length was more than double for di-ammonium phosphate and BioTRP treatments compared to control. The number of grains/panicle was also highest for di-ammonium phosphate and BioTRP1 with 54 and 51 grains/panicle respectively. The di-ammonium phosphate, the BioTRP1 and control were significantly different and showed the maximums and minimum weights of 28; 27 and 20 g for 1000 grains of wheat. BioTRP2 and BioTRP3, formulated with

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Thiobacillus thiooxidans AHB411 and Thiobacillus thiooxidans AHB417, also caused yield increases of 33.33 and 11.97% respectively. The choice of Thiobacillus thiooxidans AHB 436, and the formulation of BioPNT will be discussed

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Keywords: Tilemsi Rock Phosphate, Thiobacillus, Phosphate solubilization, Wheat, Mali.

1. Introduction

Wheat is one of the fastest growing food sources in the Northern Mali, mainly in the Timbuktu region [1]. Wheat plays an important role both in food security and economic prosperity of farmers in the region of Timbuktu. But wheat yield are decreasing from year to year because of soil mineral deficiencies. It is urgent to improve wheat yield in this region. Improving wheat production is not only important for food security but also for the livelihoods of rural producers. An essential nutrient to increase wheat production is phosphorus (P) [2]. Mali has deposits of rock phosphate (RP), an inexpensive source of phosphorus. Soluble P fertilizers can be strongly adsorbed by soil minerals and (or) precipitated with free ions in the soil solution (Al3+, Fe2+, Ca2+) [3]. Because of this problem, high rates of P fertilizers are required to achieve normal plant growth [2]. Unfortunately, this is very expensive and not always affordable in many underdeveloped countries, as Mali [4]; [5]. A viable alternative is the use of locally available rock phosphates (RP). However, the effectiveness of most of these materials is limited by their very low dissolution rate [6] and conduct to the use of high quantity of RP. The quantity of RP required can be substantially reduced by increasing its dissolution rate [4]. One of the biological approaches of achieving this objective is through the use of P-solubilizing bacteria (PSB) [1]; [7] ; [8]; [9] and [10]. These bacteria are able to improve plant growth by solubilizing sparingly soluble inorganic and organic phosphates in the soil. Oxidizing sulfur and sulfide compounds and release sulfate is an important mechanism involved in inorganic P solubilization [11]. Thiobacillus thioparus, Thiobacillus thiooxidans and Thiobacillus ferrooxidans are considered for being able to transform sulfurated compounds with reduced valence in soils and influence phosphorus's availability to plants [11] and [12]. Thiobacillus thiooxidans strains isolated in Mali can make soluble the phosphorus from the Tilemsi rock phosphate by producing sulfuric acid from the oxidation of elementary sulfur and of some sulfide [11]. The capacity of these bacteria to produce sulfuric acid from the oxidation of sulfurs or sulfides can be exploited to formulate biological phosphate fertilizer from Tilemsi Rock phosphate [13]. The peculiarity of this fertilizer would be that the phosphorus would be made available by the biological oxidation of the sulfur contained in the fertilizer. The oxidation of sulfur in soil, being a biological process, the solubilization of the natural rock phosphate depends on the nature of the sulfur and the sulfur-oxidizing bacteria strains. As, sulfur oxidation in soil vary with the quantity of P-solubilizing microorganisms in soil, we hypothesis that, by formulated biofertilizer containing these microorganisms and using them as P sources, we can improve natural rock phosphate as low cost P fertilizer and enhance crop yield in Africa. The aim of this study was to formulate a low cost and easy to use BioTRP and evaluate it efficiency as P fertilizer for wheat cultivated in Mali.

2. Material and Methods

2.1. Bacterial strains

Thiobacillus thiooxidans AHB436, *Thiobacillus thiooxidans* AHB411 and *Thiobacillus thiooxidans* AHB717 used in this study was isolated from Malian agricultural soils [1] and [11], and maintained in the LaboREM-Biotech microorganism culture collection in Mali. These bacteria have been selected for their: (i) high P-solubilizing activities measured in liquid media containing TRP as sole source or phosphorus and elemental sulfur or sulfides as energy source and (ii) capacity to improve crop growth in greenhouse studies [11].

2.2. Tilemsi rock phosphate (TRP)

The TPR deposits contain between 23 and 32% of P2O5 and their solubility in neutral ammonium citrate is 4.2% [14]. The fine TPR powder used had the following composition (in mg g^{-1}): P, 150; Ca, 329; Al, 20; F, 29. The extractability of P from TPR determined according to [15] was 16.2 mg g^{-1} in 2% citric acid and 73.4 mg g^{-1} in 2% formic acid.

2.3. Formulation of phosphate biofertilizers

2.3.1. Bacterial inoculum

To produce bacterial inoculum, the three thiobacilli strains used in this project was grown in a 300 ml Erlenmeyer flasks containing liquid medium [16] composed with: (NH4)2SO4, 0.4g; CaCl2, 0.20g; KH2PO4, 3g; MgSO4.7H2O, 0.5g; FeSO.7H2O, 0.01g; So, 10g 1% thiosulfate and distilled water 1000 ml with the pH of the medium adjusted at 4 for *Thiobacillus thiooxidans* (AHB 411 and AHB 436) strains and 7 for *Thibacillus thioparus* (AHB717). The flasks were loosely capped and shaken at 225 rpm at room temperature for 48 hours on a rotary shaker. For harvesting bacterial cells, in each culture broth (10 ml) containing *Thiobacillus* was centrifuged at 10 000 rpm for 15 min and suspended in phosphate buffer (0.01M, pH 7.0). The optical density of each *Thiobacillus* strain was adjusted to a concentration of 10^8 CFU/ml using a spectrophotometer. The bacterial inoculum was used in the in vitro selection tests and the development of bacterial based biofertilizer formulations.

2.3.2. Bio-phosphate formulation

Three biological superphosphates (″BioTRP″) was produced as described by [17]. To formulate these bio-phosphates, finely ground Tilemsi rock phosphate (<150 µm) was dry mixed with finely ground sulfur (< 150 µm) at a ratio of 5:1 by weight and inoculating it with *Thiobacillus Thiooxidans* AHB436 (BioTRP1), *Thiobacillus thiooxidans* AHB411 (BioTRP2) and *Thiobacillus thioparus* AHB717 (BioTRP3). After shade drying overnight till the water holding capacity will be 3%, the different formulations were packed in polypropylene bags and sealed. The superphosphate bags were conserved at 4ºC and local temperature, and at different times a sample was removed in each bag and tested for quality (bacterial cell concentration) using the dilution plate and direct count methods.

2.4. Field experiment

Experimental plots were established in "Diré" (Mali) during the 2013–2014 cropping season. The 0–15 cm of the silty clay soil at the site had a pH of 6.37 (0.01 M CaCl2, 1:1 v/v) and contained 0.17% organic matter. [18] available elements (kg ha^{-1}) were as follows: P, 6.3; K, 240; Ca, 804; Mg, 217; Fe, 43 and Al, 255. The experimental design was carried out in complete blocks design with 4 blocks (the blocks were established perpendicular to an east-west slope present on the site d experimentation). Seven treatments including: T1=control (no P fertilizer applied), T2= di-ammonium phosphate (100 kg/ha), T3= rock phosphate (150 kg/ha), T4= rock phosphate + P solubilizing bacteria, *Pseudomonas* Sp. BR2, T5= BioTRP 1, T6= BioTRP2 and T7= BioTRP3 were randomly distributed in plots measuring 5 x 5m. To improve the organic matter statut of the experimental plots, manure was applied from T1 to T7 at 13t/ha and incorporated into the soil with a superficial tillage. The dry soil was leveled after breaking up the soil chunks. There was a 3m-space between the blocs so that any likely interaction effects would be inhibited. Experimental plots were irrigated separately to avoid cross contamination. The first irrigation was done before seeding, so that soil conditions were moist when seeded. Acidophilic strains formulations contain 7 x 10^7 cell/g of inoculum. While the formulation with the P solubilizing bacterium, *Pseudomonas* Sp. BR2, isolated from the Malian soils [19] and [1] contain 2.5 x 10^8 cell/g of inoculum. All treatments were used before planting. One seed of wheat (cv. Tetra) was sown in the middle of each pocket with a spacing of 25 cm x 25 cm between pockets. The bacterial treatments (formulations) of *Thiobacillus* and P solubilizing bacteria were used at 16 g per pocket. During the different stages of plant growth, practices such as manual weeding, irrigation and pest control were performed Plots were fertilized with urea at 75 kg/ha for all the treatments.

After harvesting the plants, grain yield calculated at 13% moisture, tillers/plant, panicles/tiller (Rice yield increases can be due to increase in the number of productive tiller and the number of spikelets per panicles), and percentage of fertile tillers (number of tillers with panicles/total number of tillers), panicle length, grain/panicle and 1000 grain weight were determined. Yield relative efficiency was calculated using the following formula:

$$\text{Yield relative efficiency} = \frac{\text{Plant dry mater in the treated plots} - \text{plant dry matter in the control}}{\text{Plant dry matter (treated with di-ammonium phosphate)} - \text{plant dry matter in the control}}$$

The data recorded for each treatment were analysed statistically using SAS software [20]. Significant differences were determined by Duncan's Multiple Comparison Test.

3. Results and discussion

3.1. Results

It was determined that the experimental soil is very poor in organic matter and a very little amount of available P for plant uptake, less than necessary for wheat production. According to the analysis of variance the effects of experimental treatments on wheat grain and straw yield and number were significant at 1% level (data not shown). The effects of the different treatments on the weight of 1000 grains, the number of tillers per plants, the percentage of fertile tillers, the number of panicles per plant, the panicle length and the number of grains per panicles were significant at 5% level (data not shown). The highest amounts of grain (3100 kg/ha) and straw (4100 kg/ha) yield were taken from treatment 2 (di-ammonium phosphate), significantly different from the control treatment with the corresponding values of 1950 and 2100 kg/ha, respectively (Table 1).

Table 1. Effects of different treatments on wheat grain yield and straw 350

Treatments	Grain yield			Straw yield		
	Kg/ha	Increase relative to the control (%)	Relative Yield Efficiency (%)	Kg/ha	Increase relative to control (%)	Relative Yield Efficiency (%)
T1	1950^{e}	-	-	2100^{f}	-	-
T2	3100^{a}	58,97	100	4100^{a}	95,24	100
T3	2300^{d}	17,94	30,43	2650^{e}	26,19	27,50
T4	2550^{c}	20,51	34,78	2990^{d}	42,38	44,50
T5	2840^{b}	45,64	77,39	3650^{b}	73,81	77,50
T6	2600^{c}	33,33	56,51	3070^{c}	46,19	48,50
T7	2180^{d}	11,97	20	2650^{e}	26,19	27,50

Values followed by the same letters are not statistically different at P= 0.05 using Dunkan's Multivariate test. (T1) control, (T2) di-ammonium phosphate (100 kg/ha), (T3) rock phosphate (150 kg/ha), (T4) phosphate + P solubilizing bacteria, (T5) BioTRP1, (T6) BioTRP2 and (T7) BioTRP3.

Treatment 5 (BioTRP1) producing 2840 kg/ha grain yield was the only treatment with a slightly different from the di-ammonium phosphate treatment. When compared to the control, treatment 5 increased grains and straw yield at 45.64 and 73.81%, respectively with the highest relative yield efficiency (Table 1). For all treatments, plants tillered remarkably higher compared to the control plants. At harvest, average number of tillers was 18 for treatment 5 and a bit lower for treatment 2 (di-ammonium phosphate) with 17.8. It was much lower for the control with only 4 tillers/plant (Table 2).

Table 2. Effects of different treatments on the number of tiller per plant and the percentage of fertile tillers

Treatments	Tillers/plant (Number)	Fertile Tillers (%)
T1	3.8	72
T2	17.8	92
T3	8	74

T4	11	83
T5	18	90
T6	15.4	86
T7	9.8	83

(T1) control, (T2) di-ammonium phosphate (100 kg/ha), (T3) rock phosphate (150 kg/ha), (T4) phosphate + P solubilizing bacteria, (T5) BioTRP1, (T6) BioTRP2 and (T7) BioTRP3.

Percentage of fertile tillers was 92% and 90% for the di-ammonium phosphate and the BioTRP1 treatments respectively, whereas for the Tilemsi rock phosphate alone and the control it was significantly lower with 74% and 72%, respectively. Panicle length between the different treatments was strikingly different. Panicle length was more than double for di-ammonium phosphate and BioTRP treatments compared to control, or 10 cm for di-ammonium phosphate, 9 cm for BioTRP1, and 4 cm for the control (Table 3).

Table 3. Effects of different treatments on the number of panicles per plant, the panicle length, the number of grains per panicle, and the weight of 1000 grains of wheat.

Treatments	Panicles/Plant (Number)	Panicle length (cm)	Grain/Panicle Number)	1000grains weight (g)
T1	2.9	4	32	20
T2	16.6	10	54	28
T3	5	6	40	22
T4	13.4	7	46	24
T5	15.2	9	51	27
T6	15	8	49	25
T7	12.9	7	44	24

(T1) control, (T2) di-ammonium phosphate (100 kg/ha), (T3) rock phosphate (150 kg/ha), (T4) phosphate + P solubilizing bacteria, (T5) BioTRP1, (T6) BioTRP2 and (T7) BioTRP3.

The number of grains/panicle was also highest for di-ammonium phosphate and BioTRP1 with 54 and 51 grains/panicle respectively, followed by BioTRP2 with 49, and the control with 32. The weight of 1000 grains ranged from 20 to 27 g, was not significantly affected by different treatments (Table 3). Treatments 2 (di-ammonium phosphate), treatment 5 (BioTRP1) and control were significantly different and showed the maximums and minimum weights of 28; 27 and 20 g for 1000 grains of wheat (table 3).

3.2. Discussion

The experimental treatments tested in this study significantly enhanced wheat yield, indicating that the right combinations of treatments have been proposed. Different scientists have been previously observed the great advantages of using plant growth promoting rhizobacteria (PGPR), including P solubilizing and S-oxidizing bacteria, to enhanced plant growth [19] and [21]. Up to date, in Mali, there is very little data related to the effects of sulfur oxidizing bacteria on wheat growth, fertilized with rock phosphate, particularly when combined with sulfur. According to the results of this experiment, di-ammonium phosphate treatment resulted in the highest amount of plant yield. Compared with rock phosphate (17.94% of yield increases), higher yield increase, 20.51% and 45.65%, was resulted when wheat plants were inoculated with P-solubilizing bacteria and sulfur oxidizing bacteria which solubilize phosphorus. In addition to enhanced P solubility through producing sulfuric acid by oxidizing sulfur and sulfides, these bacteria can improve wheat S-nutrition [22]. We examined the effects of different combinations of biological methods (biofertilizers) on wheat growth in the presence of rock phosphate. It was accordingly indicated that soil microorganisms including P solubilizing bacteria and sulfur oxidizing bacteria are very important components (biofertilizers) of the treatments tested in the experiment. As P-solubilizing bacteria, sulfur oxidizing bacteria are able to enhance P solubility of rock phosphate and hence its availability to the plant by oxidizing sulfur and hence decreasing soil pH. Using very affordable sources of P (rock phosphate) and elemental sulfur combined with the related microorganisms tested in this experiment can have very favorable economic and environmental advantages. The right combinations of rock phosphate and biological resources can greatly contribute to the enhanced wheat yield and yield components, while agriculturally sustainable.

4. Conclusion

The present research shows that: mixing rock phosphate with sulfur and inoculated it with P solubilizing bacteria and *Thiobacillus thiooxidans* resulted in higher plant growth and yield compared to the control (non-inoculated) and the treatment with rock phosphate alone. According to the results, and with respect to the presence of high amount of sulfur and rock phosphate resources in the country and other parts of the world, it is suggested that in soils with different buffering capacities, and for different crop plants different combinations of sulfur and rock phosphate sulfur oxidizing bacteria be used. To optimize the use of soluble P fertilizers, we suggest to combine them with the biofertilizers and compare the efficiencies.

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